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(54) Title: A METHOD FOR THE IMPROVEMENT OF TRANSPORT ACROSS ADAPTABLE SEMI-PERMEABLE BARRIERS

(57) Abstract: The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate; said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

5       A Method for the Improvement of Transport Across Adaptable Semi-  
Permeable Barriers

The present invention is in the field of administration of drugs and particularly drug delivery across barriers. It more particularly relates to a method for controlling the flux  
10 of penetrants across an adaptable, semi-permeable porous barrier. It further relates to a kit and a device which both enable the drug to be controllably applied.

A porous barrier as used herein is any obstacle comprising pores which are too narrow to let the penetrants diffusively pass. This necessarily implies that the penetrants are  
15 bigger than the average diameter of such a pore.

Some barriers, such as artificial porous membranes, for example ion-track polycarbonate membranes, may have permanent properties, while others are characterised by a possible change of their properties. Most notably the pore size and  
20 more rarely the pore density, may change as a function of the surroundings and/or of the flux of the penetrants through the pores in the barrier. The latter can be found with living tissues which are separated by boundaries with such properties, for example, cells and cell organelles.

25 The skin is used to further illustrate the basic principle of such a barrier:

The maximum barrier properties of the skin reside in the outermost skin region, that is, in the horny layer (*stratum corneum*). This is owing to special chemical and anatomical characteristics of the horny layer, which preclude most efficiently the  
30 passage of essentially any material across the skin.

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5 In the stratum corneum, 20-30 consecutive layers of the skin cells (chiefly corneocytes) are organised into columns. These columns are oriented perpendicular to the skin surface, permitting the cells from adjacent columns to overlap laterally and forcing the cells from one layer to be overlaid and packed densely. Intercellular junctions in the horny layer, moreover, are tightly sealed with specialised lipids, chiefly ceramides,  
10 which abound in the skin. The skin lipids are also predominantly well packed: typically, they form lipid multilamellae, which are coupled covalently to the neighbouring cell (envelope) membranes. Individual multilamellar stacks that run parallel to the cells surface are joined together with the less well ordered lipid domains. In such domains, the non-ceramide lipids (fatty acids, cholesteryl-sulphate, etc.)  
15 prevail.

The skin lipid tendency to self-arrange into densely packed, multilamellar structures is enhanced or even driven, by the hydration or certain ion (e.g.  $\text{Ca}^{2+}$ ) concentration gradients in the skin. This may explain why similar lipid organisation is not observed  
20 elsewhere in the body except, with a much lower abundance, in the oral cavity.

Chemical skin permeation enhancers, for example dimethylsulfoxide, promote the diffusion of drugs across the skin by solubilising or extracting some of the intercellular lipids from the barrier. Transcutaneous transport is therefore most efficient in the least  
25 tightly packed lipid regions, where hydrophobic pores in the barrier are created most easily. Through such pores sufficiently small and lipophilic agents can diffuse along the transcutaneous concentration gradient(s). The resulting skin permeability is unaffected by the agent concentration, unless the agent acts as an enhancer, but the permeability depends on the concentration and the selection of skin permeation  
30 enhancer(s).

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5 However the hydrophobic pores in the skin are not big enough to allow an appreciable transport of large drugs of any kind. Owing to the self-sealing tendency of the intercellular lipid domains the pores are also rather short lived. The lipophilicity of typical pores in the skin also precludes the transport of hydrophilic, that is, of highly polar, molecules across the organ. Conventional skin permeation enhancement is  
10 therefore only useful for the delivery of fatty materials which do not irritate the skin too much, the enhancer-mediated transport and irritation being poorly tolerated by the consumers in many cases.

Therefore to date, permeation based drug delivery through the skin is really  
15 successful only for small drugs with a molecular weight below 400 Da. Such drugs can partition into the intercellular lipid matrix in the skin and then diffuse through small hydrophobic pores in the horny layer, first into the skin proper and then further down towards the deep body tissues. The resulting steady state transport is preceded by a short lag-time period, during which the drug traverses the barrier. Transcutaneous  
20 transport does not suffer from the first pass effect, however.

The bioavailability of drugs delivered through the skin by such conventional means is typically below 50 %, and often does not even reach 25 % (Hadgraft, 1996; Cevc, 1997).

25 Large hydrophobic molecules normally cross the skin in negligible quantity only. As already mentioned above this is due to the lack of suitable passages in the skin. Transcutaneous transport of macromolecules therefore chiefly relies on the molecular diffusion through shunts, such as pilosebaceous units. To deliver a bulky and highly  
30 polar agent across the skin other methods than those conventionally used are therefore required. For example various skin poration techniques were introduced to create

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5 hydrophilic pores in the skin suitable for the purpose (to avoid confusion we will call such hydrophilic pores channels):

The simplest, and crudest solution, for making a wide channel through the skin is to eliminate mechanically the skin barrier. For example, to deliver a large, hydrophilic  
10 antidiuretic peptide 1-deamino-8-D-arginine vasopressin across the human skin from an occlusive patch the removal of a small piece of epidermis by vacu-suction has been used (Svedman et al., 1996):

Further, a most common method for opening a wide channel through the skin is to use  
15 an injection needle or mechanical impact(s) (injection; powderjection). Locally restricted skin challenge is also possible. This can be done by local heat application (thermoporation); by using high voltage pulses ( $>150$  V; electroporation); or by acoustic energy, such as ultrasound (few  $W\ cm^{-2}$ ; sonoporation). The resulting channel size depends on the nature and intensity of the skin treatment, but not on the nature or  
20 the applied amount of molecules to be transported.

Openings or even craters in the skin created by the above mentioned methods heal rather slowly under normal application conditions; the wider the passage, the more so. The skin thus may behave as an adaptable, but slowly recoverable barrier.

25

Even the most commonly used methods for making pores in the skin rely on gadgets plus experience for the proper operation; they also involve skin disinfection to protect the patient. This notwithstanding, their harm and inconvenience is tolerated as long as therapeutic benefit is achieved.

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5 The most recent tool for creating hydrophilic passages in those barriers, such as the skin is provided by microscopic barrier penetrants which directly and reversibly open said hydrophilic channels. Such penetrants are independent of external energy source and also do not rely on any gadgets. They are also well tolerated by the skin. Such penetrants known to date all belong to the class of highly deformable complex  
10 droplets (Transfersomes®). Such droplets adapt to the pores of the barrier - which they then cross efficiently - provided that the droplet components and preparation are properly selected and/or optimised. A sufficiently adaptable and hydrophilic droplet can therefore cross the barrier, such as skin, spontaneously. Such hydrophilic channels are opened transiently by the moving penetrant after the latter has adjusted its shape to  
15 achieve the goal. This allows the adjustable droplets to act as vehicles for the delivery of various - hydrophilic or hydrophobic - agents across the barrier.

Most useful droplets comprise an aqueous core surrounded by an highly flexible mixed lipid bilayer, which makes the aggregate ultradeformable and superficially highly  
20 hydrophilic. Both is required for an efficient transcutaneous transport (Cevc, 1997). Said droplets were demonstrated to transport their mass rather efficiently across the skin under optimum application conditions (Cevc, 1997).

Other types of aggregates (liposomes, niosomes, nanoparticles, microemulsions, etc.)  
25 also have been claimed to traverse the skin efficiently but were seldom, if ever, proven really to deliver the associated drugs across the skin in practically meaningful quantities. It is believed that in contrast to the highly deformable droplets (Transfersomes®) the used aggregates are either insufficiently deformable and/or are too unstable to achieve the goal. Conventional aggregates instead act as simple drug  
30 reservoirs on the skin: the aggregates, incapable of crossing the barrier, remain on the skin while the drug is released gradually from the 'vehicle' to then probably diffuse

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5 through the skin barrier on its own. The main action of conventional drug loaded suspensions is thus to increase the skin barrier hydration and/or to shed the molecules with the skin permeation enhancing capability into the tissue.

Contrary, the composite, ultradeformable lipid droplets (Transfersomes®) deform and  
10 then penetrate the skin rather than to coalesce locally. Such aggregates motion across the skin seems to proceed along the natural moisture gradient(s) between the skin cells, which guides the aggregates into the hydrophilic (virtual) channels in the organ.

The predecessors of those channels that let highly adaptable droplets pass through the  
15 skin are originally so narrow that they only permit evaporation of (rather small) water molecules across the skin. These originally tiny pores (diameter < 0.5 nm) seem to open reversibly, however, when the stress of partial dehydration of a droplet, which is thereby being forced into the channel mouth under non-occlusive conditions, becomes excessive. The strong hydrophilicity and the large mass of the droplet are the factors  
20 which maximise the droplets' tendency to move through the skin; however the droplet adaptability is the necessary condition for the success of said motion.

The movement of the droplets across the skin seems to proceed along the path pursued by the water molecules during the skin passage in the opposite direction. The droplets  
25 are thus guided into intercellular regions precisely at the points where the contacts between the above-cited skin sealing lipids are the weakest and the least tight. The corresponding skin region covered with the channels has been estimated to be around 4 % of the total skin area, or less.

30 It is possible to associate small and large, hydrophobic and hydrophilic molecules with ultradeformable and highly adaptable droplet-like aggregates. Using such complex

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- 5 aggregate droplets all types of molecules can thus be delivered across the barrier, such as the stratum corneum.

High systemic availabilities of the drug transported are typically achieved. Relative efficiency of the transport across the skin exceeds 50 %, in most cases (Cevc et al.,  
10 1996). The steady state is reached within few hours, by and large (Cevc et al., 1998).

It has already been proven that the skin barrier recovers fully after those droplets have been eliminated from the skin surface. In contrast, the channels created by other means, such as ultrasound remain open for at least 20 hours. In fact, they are not resealed  
15 properly before 2 days, even when relatively weak therapeutic ultrasound is used. Stronger perturbation causes more persistent skin damage (Mitragotri et al., 1995). (In the extreme case, when the barrier is eliminated by vacu-suction, the skin does not recover fully until after of 8 weeks.)

- 20 The precise size distribution of the channels in the skin, through which highly deformable droplets migrate spontaneously across the stratum corneum, is as yet unknown. It is probable, however, that it is asymmetric. The average width, that is, the distribution maximum has been estimated to be 20-30 nm under typically used application conditions. The skewed distribution could result from the existence of two  
25 quantitatively different but qualitatively similar intercellular transport routes across the skin (Schätzlein & Cevc, 1998) which together form the family of transcutaneous pathways.

The first, inter-cluster pathway leads between the groups of corneocytes. It represents  
30 the high-end tail of channel-size distribution and typically starts at the bottom of inter-



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5 cluster gorges. From here, it follows the dense material filling such gorge and offers the lowest resistance to penetration at the junctions where several clusters meet.

The second, intra-cluster pathway leads between the individual corneocytes in each cluster of corneocytes. This route typically proceeds along the lipid layers surface. In  
10 the projection over the outer third of the stratum corneum, the inter-corneocyte pathway resembles an interwoven three-dimensional network including all the cells in the organ. (Schätzlein & Cevc, 1998).

The above mentioned distinctions are quantitative in nature. No doubt exists that  
15 transcutaneous channels with the exception of pilosebaceous units are resistant to the passage of non-deformable, large aggregates.

Channel properties are also sufficiently constant to reveal little inter-site, inter-individual, inter-species or inter-carrier variability. According to the prior art, the  
20 relative bio-availability of different drugs in the blood after an epicutaneous administration in highly adaptable droplets (Transfersomes®) is fairly constant (Cevc, 1997). Pore distribution depends little on the nature of the penetrant or the drug. The same has been implied for the dose dependence, which was concluded to affect merely the depth of penetrant and drug distribution. Small dose per area was found to favour  
25 the local (superficial) retention whereas a large dose per area was shown to ensure a relatively great systemic availability.

Surprisingly, and contrary to the above-mentioned conclusion, we have now found out that changing the applied dose above a certain threshold and in sufficiently wide range  
30 not only affects the drug/penetrant distribution, but also determines the rate of penetrant transport across the barrier.

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- 5 Our new and unexpected finding provides means for controlling the rate of transcutaneous drug delivery whenever highly deformable carriers are used on the barrier; it also provides the basis for better, i.e. more rational, design of the delivery device. There will especially be profit for the development of cutaneous patches suitable for the use in combination with highly adaptable carriers (Transfersomes®).
- 10 Improved therapy and higher commercial value of the products should be the consequence.

It stands to reason that the observed new effect reflects the widening of channels in the barrier, but the applicant does not wish to be bound to this hypothesis. The newly

15 found dosage-dependent pore widening is probably different for various transcutaneous channels: the originally narrower pores probably change more than the relatively wide (e.g. inter-cluster) channels. The effect of relative channel size, that is, of channel vs. penetrant size ratio, suggests that it will take much longer time to bring certain penetrants quantity through narrow than through wide channels.

- 20
- If the channels act as transported mass discriminators, and adjust their width to the flux requirement, the narrow channels will persist much longer in their original, high penetration resistance state than the wide channels. However, after having responded to the multi-penetrant passage by increasing their width such channels will start to
- 25 behave as the originally wider channels. Multiple adjustments are possible but only to certain upper limit.

Another potentially important factor acting in the same direction is the skin surface hydration, which is prone to increase with enlargement of the topically administered

30 dosage. Similar mechanism has been proposed to explain the effect of conventional lipid suspension (liposomes) on the diffusive transport of large entities into the skin

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5 (Cevc, 1997). In either case, the average width and the size distribution of channels in the skin will shift towards greater values with increasing applied dosage. This then will result in higher final transcutaneous flux.

For the avoidance of doubt, all pertinent information, definitions and lists from the  
10 previous patent applications of the same applicant are incorporated herein by reference.

Kits and more particularly devices for administering drugs through a barrier such as skin or mucosa have also already been described. These devices can typically be divided into matrix systems and liquid reservoir systems. They  
15 are commonly in the form of a laminated composite that includes a reservoir layer containing the drug, a pressure sensitive adhesive layer for attaching the composite to the skin, and a backing layer that forms the upper layer of the device. Depending upon the particular drug and drug formulation involved, the reservoir layer may be a matrix in which the drug formulation  
20 is dispersed or a layer in the form of walled container which holds the drug formulation. Container-type reservoirs are often formed as a pocket between the backing layer and a drug-permeable basal membrane through which the drug passes to the skin. The pressure sensitive adhesive layer normally underlies the membrane and the drug also passes through it on its way to the  
25 skin.

Matrix-type transdermal patches are those in which the drug is contained in and released from a polymer matrix. The matrix is typically made of a pressure sensitive adhesive and defines the basal surface of the patch (i.e.  
30 the surface affixed to the skin).

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5 A number of reservoir and matrix type systems have been described.

US-Patent No. 829,224 to Chang et al., for instance, discloses a device with a reservoir that is defined by a backing layer and a drug-permeable membrane layer. A ring-shaped layer made of an adhesive is peripheral to the reservoir. A peelable liner layer underlies the membrane. A second peelable layer, the release liner, underlies the entire assembly. A first heat seal connects the backing layer and the membrane and surrounds the reservoir. A second heat seal concentric about the first heat seal connects the backing layer and the release liner. The second heat seal is broken when the release liner is removed. The device may include an inner liner that underlies the membrane and portions of the backing layer. This inner liner is removed following removal of the release liner so that the membrane is exposed.

20 U.S.-Patent Nr. 4,983,395 to Chang et al., relates to another device with a backing layer and a membrane layer that define a reservoir. A peelable inner liner underlies the reservoir and portions of the backing and membrane layers outside the periphery of the reservoir. An adhesive layer underlies the inner liner and remaining portions of the backing and membrane layers. A peelable release liner underlies the adhesive layer. A first heat seal connects the backing and membrane layers on the periphery of the reservoir. A second heat seal underlies the first heat seal and connects the membrane and the inner liner. In use, the release liner and inner liner are peeled away to expose the undersurfaces of the membrane and adhesive layers prior to placement of the device onto the skin or mucosa.

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5 PCT-Application W096-19205 to Theratech, Inc., discloses a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of an adhesive overlay, a backing layer underlying the central portion of the adhesive overlay, an active agent-permeable membrane, the backing layer and membrane defining a reservoir  
10 that contains a formulation of the active agent, a peel seal disc underlying the active agent-permeable membrane, a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer and a removable release liner underlying the exposed overlay and peel seal disc. The adhesive layer is above and peripheral to the path of the active  
15 agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. The peel seal disc protects against release of the active agent-containing reservoir and the release liner protects the adhesive from exposure to the environment prior to use.

20 US-Patent No. 5,202,125 to Theratech, Inc., describes a transdermal delivery system for delivery of nitroglycerin which deliver the drug at enhanced transdermal fluxes. The systems include, in addition to nitroglycerin, a permeation enhancer which is either a sorbitan ester, a C8-  
25 C22 aliphatic alcohol, or a mixture thereof. Methods for administering nitroglycerin using such permeation enhancers are also disclosed.

WO90-11065 to Theratech, Inc., discloses a transdermal drug delivery device comprising a drug formulation containing reservoir defined by a  
30 backing layer and a drug-permeable membrane layer, a peelable inner liner that underlies the reservoir and a portion of the backing/membrane

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5 outwardly of the reservoir periphery, an adhesive layer that underlies the  
inner liner and outwardly extending portions of the membrane/backing  
layers, and a peelable release liner layer that underlies the adhesive layer  
with a first permanent heat seal between the backing and the membrane  
about the perimeter of the reservoir and another peelable (impermeant) heat  
10 seal between the membrane and the inner liner underlying the first  
permanent heat seal, the heat seals and peelable barrier layer providing  
barriers that isolate the drug formulation from the adhesive.

US-Patent No. 5,460,820 to Theratech, Inc., discloses a method of providing  
15 testosterone replacement therapy to a woman in need of such therapy  
comprising applying a testosterone-delivering patch to the skin of said  
woman which patch transdermally delivers 50 to 500 µg/day testosterone to  
the woman. The skin patch comprises a laminated composite of a backing  
layer and a matrix layer comprising a solution of testosterone in a polymeric  
20 carrier, said matrix layer providing a sufficient daily dose of testosterone to  
provide said therapy.

US-Patent No. 5,783,208 to Theratech, Inc., discloses a matrix-type  
transdermal patch for coadministering estradiol and another steroid wherein  
25 the matrix is composed of a N-vinyl-2-pyrrolidone-containing acrylic  
copolymer pressure sensitive adhesive, estradiol the other steroid, and  
optionally a permeation enhancer, and the respective fluxes of estradiol and  
the other steroid from the matrix are independent of the respective  
concentrations of the other steroid and estradiol in the matrix.

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- 5 All pertinent information, definitions and lists from the patents and patent applications of the US-company Theratech, Inc. are expressively incorporated herein by reference.

10 It is an important object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier, such as the skin of a human or animal body or a plant. It is another object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier in using a kit or device which enables the  
15 formulation to be applied at the selected dose per area.

According to the present invention this is achieved by a method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:

- 20 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
- 25 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
- and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the  
30 diameter of homo-aggregates containing merely the less soluble substance,

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- 5    – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher;
- and / or the presence of the more soluble substance lowers the average  
10   elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said  
15   barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- 20   – applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

      Preferrably the flux of penetrants across said barrier is increased by enlarging the applied dose amount of said penetrants.

- 25    It then is preferred if the pH of the formulation is between 3 and 10, more preferably is between 4 and 9, and most preferably is between 5 and 8.

- According to another preferred feature of the present invention the  
30   formulation containing the penentrants comprises:



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- 5    – at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- 10    – and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- 15    – and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 4 days.

It then is preferred if said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass

20    of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

25    It then is also preferred if said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates,

30    poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-

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- 5    sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,  
polyethylene glycol-lactides, polyethylene glycol-diacrylates,  
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),  
poly(propylene fumarate-co-ethylene glycols), poloxamers,  
polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;  
10    natural gums comprising alginates, carrageenans, guar-gums, gelatines,  
tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;  
mixtures and further derivatives or co-polymers thereof and / or other  
pharmaceutically, or at least biologically, acceptable polymers.
- 15    Preferably the concentration of said polymer is chosen to be in the range  
between 0.01 w- % and 10 w- %, more preferably in the range between 0.1  
w- % and 5 w- %, even more preferably in the range between 0.25 w- % and  
3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.
- 20    Further it is preferred that said anti-oxidant is selected from synthetic  
phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated  
hydroxytoluene (BHT ) and di-tert-butylphenol (LY178002, LY256548,  
HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.),  
tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-  
25    trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine,  
p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol,  
tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol,  
hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid,  
quinic acid, syringic acid, ellagic acid , salicylic acid, nordihydroguaiaretic  
30    acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta,  
gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.

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- 5 -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.);
- 10 non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital,
- 15 acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium
- 20 metabisulphite, thiourea; chelating agents, such as EDTA, GDTA; desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less
- 25 complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon,
- 30 sage, rosemary; mace, oregano, allspice, nutmeg); carnolic acid, carnosol,

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5 carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid;  
oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers,  
sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives  
(e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine,  
3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation  
10 suppressing enzyme.

Then, the concentration of BHA or BHT is often chosen to be between  
0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most  
preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between  
15 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most  
preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005  
and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most  
preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between  
0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably  
20 is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5,  
more preferably is between 0.005 and 0.5 w-%, and most preferably is  
between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is  
between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and  
most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001  
25 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably  
is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is  
between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most  
preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of  
monothioglycerol is between 0.01 and 5 w-%, more preferably is between  
30 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically  
0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between

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- 5 0.001–0.2 w-%, and most preferably is between 0.005–0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most  
10 preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01–0.2, most typically between 0.3 and 2 w-%.
- 15 Furthermore it is preferred if said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially  
20 alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide,  
25 cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibioticly active compounds of biological origin, or a mixture thereof.
- 30 Preferably the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up

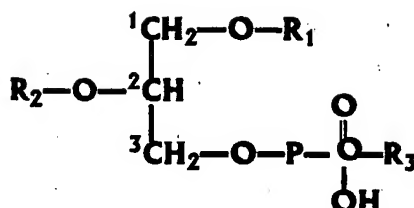
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5 to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the  
10 range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

15 It is preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material; especially a polar lipid; whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is  
20 comparably soluble as said surfactant or the surfactant-like material.

Preferrably the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure  
25 phospholipids corresponding to the general formula

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where  $\text{R}_1$  and  $\text{R}_2$  is an aliphatic chain, typically a  $\text{C}_{10-20}$ -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where  $\text{R}_3$  is hydrogen,

10 2-trimethylamino-1-ethyl, 2-amino-1-ethyl,  $\text{C}_{1-4}$ -alkyl,  $\text{C}_{1-5}$ -alkyl substituted with carboxy,  $\text{C}_{2-5}$ -alkyl substituted with hydroxy,  $\text{C}_{2-5}$ -alkyl substituted with carboxy and hydroxy, or  $\text{C}_{2-5}$ -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also

15 glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids,

20 phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-

25 groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

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- 5 The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-
- 10 aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
- 15 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-
- 20 lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-
- 25 or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-
- 30 glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,



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- 5 or -phosphorylserine, n-tetradecyl- glycerophosphatidic acid, -  
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,  
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain  
phospholipid, or else a surface-active polypeptide.
- 10 According to a preferred feature of the present invention, the average  
diameter of the penetrant is between 30 nm and 500 nm, more preferably  
between 40 nm and 250 nm, even more preferably between 50 nm and  
200 nm and particularly preferably between 60 nm and 150 nm.
- 15 It is another preferred feature of the present invention that the total dry  
weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of  
total formulation mass, more preferably is between 0.1 w-% and 30 w-%,  
and most preferably is between 0,5 w-% and 20 w-%.
- 20 It is preferred that the total dry weight of droplets in a formulation is  
selected to increase the formulation viscosity to maximally 5 Nm/s, more  
preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that  
formulation spreading-over and drug retention at the application area is  
enabled.
- 25 According to the present invention is preferred if at least one edge-active  
substance or surfactant and/or at least one amphiphilic substance, and / or at  
least one hydrophilic fluid and the agent are mixed, if required separately, to  
form a solution, the resulting (partial) mixtures or solutions are then
- 30 combined subsequently to induce, preferably by action of mechanical energy  
such as shaking, stirring, vibrations, homogenisation, ultrasonication,

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5 shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

Preferrably this amphiphilic substances are dissolved in volatile solvents,  
10 such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO<sub>2</sub>, which are then removed,  
15 especially by evaporation or dilution, prior to making the final preparation.

According to the present invention the formation of said penetrants  
preferrably is induced by the addition of required substances into a fluid  
phase, evaporation from a reverse phase, by injection or dialysis, if  
20 necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

25 Then the formation of said penetrants preferrably is induced by filtration, the filtering material having pores sizes between 0.01  $\mu\text{m}$  and 0.8  $\mu\text{m}$ , more preferably between 0.02  $\mu\text{m}$  and 0.3  $\mu\text{m}$ , and most preferably between 0.05  $\mu\text{m}$  and 0.15  $\mu\text{m}$ , whereby several filters may be used sequentially or in parallel.

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- 5 According to the invention said agents and penetrants preferably are made to associate, at least partly,
- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol
  - 10 (molecular weight: 200-400 D) or glycerol into the suspending medium,
  - simultaneously with penetrant formation, if required using the drug co-solution and; at least some, penetrant ingredients.

It is preferred if said penetrants, with which the agent is associated are  
15 prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

The formulation according to the invention preferably is applied by spraying, smearing, rolling or sponging on the application area, in particular  
20 by using a metered sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

It is preferred if the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium  
25 and / or nasal or any other mucosa.

The area dose of said penetrant then preferably is between 0.1 mg per square centimetre ( $\text{mg cm}^{-2}$ ) and 40  $\text{mg cm}^{-2}$ , more preferably is between 0.25  $\text{mg cm}^{-2}$  and 30  $\text{mg cm}^{-2}$  and even more preferably is between  
30 0.5  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$ , in the case that the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

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5 The area dose of said penetrant then preferably is between 0.0001 mg per square centimetre ( $\text{mg cm}^{-2}$ ) and 0.1  $\text{mg cm}^{-2}$ , more preferably is between 0.0005  $\text{mg cm}^{-2}$  and 0.05  $\text{mg cm}^{-2}$  and even more preferably is between 0.001  $\text{mg cm}^{-2}$  and 0.01  $\text{mg cm}^{-2}$ , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

10

The area dose of said penetrant then preferably is between 0.05 mg per square centimetre ( $\text{mg cm}^{-2}$ ) and 20  $\text{mg cm}^{-2}$ , more preferably is between 0.1  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$  and even more preferably is between 0.5  $\text{mg cm}^{-2}$  and 10  $\text{mg cm}^{-2}$ , in the case that the penetrant is applied on

15

said nasal or other mucosa.

In another advantageous aspect of the invention, a kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area as afore-mentioned is provided.

20

It then is preferred if the formulation is contained in a bottle or any other packaging vessel.

The kit preferably contains a device for administering the formulation,

25 which preferably is a non-occlusive patch.

According to another aspect of the present invention a device is provided comprising a non-occlusive patch, containing the formulation in an amount that yields the dose per area as mentioned above.

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- 5 According to the invention a non-occlusive patch comprises a laminated composite of:
- a backing layer;
  - an active agent-permeable membrane, the backing layer and membrane defining
- 10 – a reservoir therebetween that contains the formulation of the active agent, said reservoir having a smaller periphery than the backing layer and membrane such that a portion of the backing layer and membrane extends outwardly of the periphery of the reservoir;
- a pressure sensitive adhesive layer that underlies and covers the active
- 15 agent-permeable membrane and said outwardly extending portion of the backing layer and membrane.

According to the invention a non-occlusive patch also comprises a laminated composite of:

- 20 – a backing layer;
- a matrix layer that contains the formulation of the active agent; and
  - a pressure sensitive adhesive layer.

It then is preferred if the formulation and / or agent and / or

25 suspension / dispersion of penetrants without the agent are kept during the storage in several; more preferably less than 5, even more preferably in 3, and most preferred in less than 3 separate inner compartments of the device which, in case, are combined prior to or during the application of the formulation.

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5     Preferably said compartment(s) filled with the formulation and / or agent and / or suspension of penetrants without the agent, is (are) covered, on one or both sides, with a non-occlusive, semi-permeable membrane that lets small molecules, such as water, but only few or not the penetrants pass.

10    It is preferred that said non-occlusive, semi-permeable membrane is the same or different, if it is used on both sides of said device.

Accordingly it is preferred if said water permeability of said semi-permeable membrane is at least  $10 \text{ mg cm}^{-2} \text{ h}^{-1}$ , more preferably exceeds  $50 \text{ mg cm}^{-2} \text{ h}^{-1}$   
15    and most preferably is greater than  $100 \text{ mg cm}^{-2} \text{ h}^{-1}$ .

The area of said semi-permeable membrane preferably is between  $0.5 \text{ cm}^2$  and  $250 \text{ cm}^2$ , more preferably is between  $1 \text{ cm}^2$  and  $100 \text{ cm}^2$ , even more preferably is between  $2 \text{ cm}^2$  and  $50 \text{ cm}^2$  and most preferred is between  $4 \text{ cm}^2$   
20    and  $25 \text{ cm}^2$ .

It is preferred if the area of said semi-permeable membrane is the area substantially covered by the formulation filled part of the device.

25    It further is preferred if the penetrant flux across the barrier is controlled by the permeability of, or the suspension-medium evaporation across, the semi-permeable, non-occlusive membrane.

According to a preferred feature of the invention the device is filled with the  
30    formulation and / or agent molecules and / or suspension of penetrants without agent, either separately or together, prior to the administration of

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5    said patch, preferably 360 min, more preferably 60 min, even more  
      preferably 30 min and most preferably within few minutes before placing  
      the device on the barrier.

10    It another important aspect of the present invention, a method is provided of  
      administering an agent onto a mammalian body or a plant, for transporting  
      said agent through a barrier, such as the intact skin/mucosa or cuticle,  
      respectively, when the agent is associated with the penetrant which is  
      capable of transporting said agent through the skin pores or through the  
15    passages in mucosa or cuticle, or else is capable of enabling agent  
      permeation through skin pores after said penetrant has opened and/or  
      entered said pores, comprising the steps of:

- 20    – preparing a formulation by suspending or dispersing said penetrants in a  
      polar liquid in the form of fluid droplets surrounded by a membrane-like  
      coating of one or several layers, said coating comprising at least two  
      kinds or forms of amphiphilic substances with a tendency to aggregate,  
      provided that
- 25    – said at least two substances differ by at least a factor of 10 in solubility in  
      said polar liquid,
- and / or said substances when in the form of homo-aggregates (for the  
      more soluble substance) or of hetero-aggregates (for any combination of  
      both said substances) have a preferred average diameter smaller than the  
      diameter of homo-aggregates containing merely the less soluble  
      substance,
- 30    – and / or the more soluble substance tends to solubilise the droplet and the  
      content of such substance is to up to 99 mol-% of solubilising

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- 5 concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
- and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more
- 10 than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,
- 15 - selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

20

It then is preferred if the flux across said barrier is increased by enlarging the applied dose amount of said penetrants per area of barrier.

The pH of the formulation preferably is chosen to be between 3 and 10,  
25 more preferably is between 4 and 9, and most preferably is between 5 and 8.

In this aspect of the invention, it then is preferred if the formulation comprises:

- at least one thickening agent in an amount to increase the formulation
- 30 viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most



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- 5 preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per
- 10 12 months
- and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*
- 15 or *Staphilococcus aureus*, after a period of 4 days.

Said at least one microbicide then preferably is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than

20 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

Said thickening agent preferably is selected from the class of

25 pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-,

30 poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,

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- 5 polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones; natural gums comprising alginates, carrageenans, guar-gums, gelatines, 10 tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.

- The concentration of said polymer then preferably is chosen to be in the 15 range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

- 20 According to the invention said anti-oxidant then preferably is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate 25 (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, 30 nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as

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- 5    tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or
- 10    linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine,
- 15    azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol);
- 20    sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferal; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase,
- 25    glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters,
- 30    caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace,

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5 oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic  
10 acid), and organochalcogenides, or else is an oxidation suppressing enzyme. It then is preferred if the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most  
15 preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5,  
20 more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2; and most preferably  
25 is between 0.001-0.01 w-%, most typically 0.005 w-%, of cysteine is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically  
30 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most

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5 typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and  
10 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

Preferably said microbicide is then selected amongst short chain alcohols,  
15 such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben,  
20 benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as  
25 phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibioticly active compounds of biological origin, or a mixture thereof.

It then is preferred that the bulk concentration of short chain alcohols in the  
30 case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between

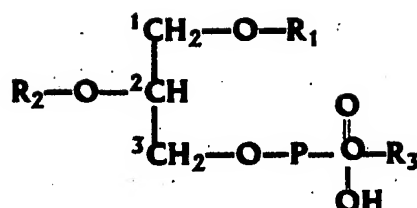
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5 0.5-3 w-%, and in the case of chlorobutanol is in the range between  
0.3-0.6 w-%; bulk concentration of parabens, especially in the case of  
methyl paraben is in the range between 0.05-0.2 w-%, and in the case of  
propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration  
of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of  
10 benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of  
phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk  
concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

It then is also preferred that the less soluble amongst the aggregating  
15 substances is a lipid or lipid-like material, especially a polar lipid, whereas  
the substance which is more soluble in the suspending liquid and which  
lowers the average elastic energy of the droplet is a surfactant or else has  
surfactant-like properties and / or is a form of said lipid or lipid-like material  
which is comparably soluble as said surfactant or the surfactant-like  
20 material.

Preferably the lipid or lipid-like material is a lipid or a lipoid from a  
biological source or a corresponding synthetic lipid or any of its  
modifications, said lipid preferably belonging to the class of pure  
25 phospholipids corresponding to the general formula

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5

where  $\text{R}_1$  and  $\text{R}_2$  is an aliphatic chain, typically a  $\text{C}_{10-20}$ -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyll-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where  $\text{R}_3$  is hydrogen,

10 2-trimethylamino-1-ethyl, 2-amino-1-ethyl,  $\text{C}_{1-4}$ -alkyl,  $\text{C}_{1-5}$ -alkyl substituted with carboxy,  $\text{C}_{2-5}$ -alkyl substituted with hydroxy,  $\text{C}_{2-5}$ -alkyl substituted with carboxy and hydroxy, or  $\text{C}_{2-5}$ -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also

15 glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids,

20 phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-

25 groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

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5 The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-  
10 aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl  
15 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-  
20 lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-  
25 or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-  
30 glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,



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- 5 or -phosphorylserine, n-tetradecyl- glycerophosphatidic acid, -  
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,  
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain  
phospholipid, or else a surface-active polypeptide.
- 10 The average diameter of the penetrant preferably is between 30 nm and  
500 nm, more preferably between 40 nm and 250 nm, even more preferably  
between 50 nm and 200 nm and particularly preferably between 60 nm and  
150 nm.
- 15 The total dry weight of droplets in a formulation is then preferably chosen  
to range from 0.01 weight-% (w-%) to 40 w-% of total formulation mass,  
more preferably is between 0.1 w-% and 30 w-%, and most preferably is  
between 0,5 w-% and 20 w-%.
- 20 Preferably the total dry weight of droplets in a formulation is selected to  
increase the formulation viscosity to maximally 5 Nm/s, more preferably up  
to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation  
spreading-over and drug retention at the application area is enabled.
- 25 Preferably at least one edge-active substance or surfactant and/or at least  
one amphiphilic substance, and / or at least one hydrophilic fluid and the  
agent are mixed, if required separately, to form a solution, the resulting  
(partial) mixtures or solutions are then combined subsequently to induce,  
preferably by action of mechanical energy such as shaking, stirring,  
30 vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,

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- 5 or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

It also is preferred if said amphiphilic substances then are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other  
10 pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol; other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO<sub>2</sub>, which are then removed, especially by evaporation or dilution, prior to making the  
15 final preparation.

The formation of said penetrants then preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical  
20 stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

It then is also preferred if the formation of said penetrants is induced by  
25 filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

30 Said agents and penetrants are made to associate, at least partly,

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- 5    - after the formation of said penetrants, e.g. after injecting a solution of the  
      drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and  
      2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol  
      (molecular weight: 200-400 D) or glycerol into the suspending medium,  
      - simultaneously with penetrant formation, if required using the drug  
10    co-solution and, at least some, penetrant ingredients.

It then is preferred if said penetrants, with which the agent is associated, are  
prepared immediately before the application of the formulation, if  
convenient, from a suitable concentrate or a lyophilisate.

15

Accordingly the formulation is applied by spraying, smearing, rolling or  
sponging on the application area, in particular by using a metered sprayer,  
spender, roller or a sponge, or a non-occlusive patch, as appropriate.

- 20   It further is preferred if the barrier is skin or at least partly keratinised  
      endothelium and / or nasal or any other mucosa.

- The area dose of said penetrant then preferably is between 0.1 mg per  
square centimetre ( $\text{mg cm}^{-2}$ ) and 40  $\text{mg cm}^{-2}$ , more preferably is between  
25   0.25  $\text{mg cm}^{-2}$  and 30  $\text{mg cm}^{-2}$  and even more preferably is between  
      0.5  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$ , in the case that the penetrant is applied on  
      said skin and / or said at least partly keratinised endothelium.

- The area dose of said penetrant preferably is between 0.05 mg per square  
30   centimetre ( $\text{mg cm}^{-2}$ ) and 20  $\text{mg cm}^{-2}$ , more preferably is between  
      0.1  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$  and even more preferably is between

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- 5    0.5 mg cm<sup>-2</sup> and 10 mg cm<sup>-2</sup>, in the case that the penetrant is applied on said nasal or other mucosa.

The area dose of said penetrant preferably is between 0.0001 mg per square centimetre (mg cm<sup>-2</sup>) and 0.1 mg cm<sup>-2</sup>, more preferably is between

- 10   0.0005 mg cm<sup>-2</sup> and 0.05 mg cm<sup>-2</sup> and even more preferably is between 0.001 mg cm<sup>-2</sup> and 0.01 mg cm<sup>-2</sup>, in the case that the penetrant is applied on plant body, plant leaves or plant needles.

- It is preferred if the method is used for generating an immune response on a  
15   human or other mammal by vaccinating said mammal.

It is preferred if the method is used for generating a therapeutic effect in a human or other mammal.

- 20   According to the present invention the above mentioned method is preferably used for the treatment of inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia,  
25   macroglobulinaemia, trombocytopenic purpura, furthermore, for the management of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia; connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves'  
30   ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as

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5 inflammatory bowel disease, nausea and oesophageal damage, for  
hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis),  
for Kawasaki disease, myasthenia gravis, various pain syndromes, such as  
postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory  
disorders, such as asthma, for the management of rheumatoid disease and  
10 osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema,  
erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis,  
pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

Without any limitation of the scope of the present invention as defined by the attached  
15 claims the invention shall now be described in more detail by referring to the following  
examples and figures only showing non-limiting embodiments of the present invention.

#### **General experimental set-up and sample preparation**

20  
**Test formulation.** Highly adaptable aggregate droplets used within the framework of  
this work had the form of (oligo)bilayer vesicles. Typically, the test formulation  
contained biocompatible (phospho)lipids, such as phosphatidylcholine, and  
(bio)surfactants, such as sodium cholate or polysorbate (Tween 80). Different  
25 phospholipid/detergent ratios have been chosen to maintain or select the highest  
possible aggregate deformability.

Manufacturing was done as described in previous applications of the applicant. In  
short, a solution of phosphatidylcholine (SPC; Natterman Phospholipids, Cologne,  
30 Germany) in chloroform was labelled with the tritiated SPC (Amersham, XXX) and  
mixed with sodium cholate (Merck, Darmstadt, Germany) to obtain a

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- 5 phospholipid/detergent ratio of 3.75/1 (mol/mol). The mixture was dispersed in phosphate buffer (pH = 7.2) to yield a 10 w-% total lipid suspension.

Vesicles in the suspension were frozen and thawed three times. Subsequently, the formulation was passed under pressure through several micro-porous filters (first 200  
10 nm; then 100 nm, and finally 50 nm or 80 nm; Poretics, CA). To check the reproducibility of vesicle manufacturing, the average size of vesicles was measured with dynamic light scattering procedure and found to be in the range of 80 nm to 150 nm.

- 15 **Test animals.** Mice of NMRI strain were 8 to 12 weeks old at the time of experimentation. They had free access to standard chow and water and were kept in suspension cages in groups of 4 to 6. Prior to test formulation administration, the application area on each animals back was shaved carefully. The test preparation was administered under general anaesthesia (0.3 mL per mouse of an isotonic NaCl solution  
20 containing 0.0071 % Rompun (Bayer, Leverkusen, Germany) and 14.3 mg/mL Ketavet (Parke-Davis, Rochester, N.Y). The administration was done with a high precision pipette on the skin which was left non-occluded. Each animal was finally transferred into an individual cage where it was kept for a day. A different cage was used for each animal for at least 24 hrs. 4 animals were used per test group.

- 25 **Test measurements.** Blood samples were collected from tail end, after termination of experiment at least. In one set of experiments, the early blood sampling was done every 2 hrs. Organ samples included: liver, spleen, kidney, and skin. The latter was also inspected superficially, by taking 10 strips (using a Tesa-Film).

- 30 **Processing the organ samples** was done according to standard procedures: for  $^3\text{H}$ -measurement, a small part of each organ and 100  $\mu\text{L}$  of the carcass lysate were used to

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- 5 get the desired and quoted experimental data. These were analysed according to the standard procedures.

To determine total label recovery, the carcass of test animals was dissolved and discharged by addition of 50 mL perchloric acid

10

Recovery (% of applied activity) was determined and the recovered doses (% of applied activity per organ) as well as the total delivered amount [ $\mu\text{g}$  lipid/g organ] were calculated.

#### 15 Examples 1-5:

##### Short term administration

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

20

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

trace amount of  $^3\text{H}$ -DPPC with specific activity: 750  $\mu\text{Ci}/500\mu\text{L}$

0.9 mL phosphate buffer, pH 7.3

Duration of experiment: 8 h

- 25 Application area: 1  $\text{cm}^2$  on the upper dorsum. The various doses applied on the test area are given in the following table.

	Group 1	Group 2	Group 3	Group 4	Group 5
Applied volume [ $\mu\text{L}$ ]	1.0	5.0	7.0	15.0	30.0
Appl. lipid amount [mg]	0.10	0.50	0.75	1.50	3.00
Applied activity [cpm]	108998	544991	817486	1634972	3269943

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5 Results of test measurements are given in figures 1 to 6.

### Examples 6-8:

#### Longer term administration

10

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

0.9 mL phosphate buffer, pH 7.3

15

trace amount of  $^3\text{H}$ -DPPC with specific activity: 250  $\mu\text{Ci/mL}$

Duration of experiment: 24 h.

Application area: 1 cm squared; dose per area is given in the following table.

	Group 6	Group 7	Group 8
Applied volume [ $\mu\text{L}$ ]	10.0	50.0	100.0
Appl. lipid amount [mg]	1.00	5.00	10.00
Applied activity [cpm]	145599	727997	1E+06

20

To test the effect of changing administered dose per area over longer period of time, even greater suspension volumes were applied on upper back of test mice.

Resulting data are analysed and presented together with those from previous experimental series in figures 1 to 7.

25

Figure 1 shows the recovery of relative activity (penetrant amount) in different layers of the skin as a function of applied activity (dose).



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- 5 Figure 2 shows the amount of carrier derived radioactivity ( $^3\text{H}$ -DPPC) in the blood as a function of time and epicutaneously administered penetrant quantity, expressed as percentage of applied dosage. As can be seen in this figure the relative amount of non-invasively administered lipid found in the blood reaches appreciable level after a clear lag-time of approximately 4 hours, but is nearly independent of the dose used.
- 10 Figure 3 indicates the relative accumulation of carrier derived radioactivity in various organs at two different time points after an increasing mass of ultradeformable carriers has been administered on the skin. It is apparent that whereas the relative amount of the carrier derived radioactivity decreases with the applied dosage at both times of exploration, the phospholipid amount in the blood, viable skin and liver in parallel
- 15 increases at  $t = 8$  h, but remains nearly unchanged at  $t = 24$  h.

Figure 4 shows the absolute penetrant distribution profile (in arbitrary units) in different layers of the skin as a function of applied activity (dose). Little dose dependence is seen in the horny layer for area doses between  $0.5 \text{ mg cm}^{-2}$  and up to  $1.5 \text{ mg cm}^{-2}$ , but greater penetrant amounts are deposited much more efficiently in the barrier. This is true 8 hours as well as 24 hours after the suspension administration. Viable skin accumulates the penetrant derived material in a dose dependent fashion in entire investigated range.

20

- 25 Figure 5 shows the total amount of penetrant recovered in different tissues (skin, blood, liver) at different times after the administration of an increasing quantity of ultradeformable penetrants on the skin grows with the applied dose per area. However, while at  $t = 8$  h, an apparent saturation tendency is observed for doses greater than  $1.5 \text{ mg cm}^{-2}$ , at  $t = 24$  h the dose dependence is linear.

5 Figure 6 shows the time dependence of penetrant derived radioactivity in the blood as a function of epicutaneously administered suspension volume (lipid amount). As can be seen from this figure the temporal penetration characteristics are essentially independent of the applied dose: after a lag-time period of 4-6 hours, nearly steady state situation is observed.

10

Figure 7 shows the penetrant derived radioactivity in the blood as a function of epicutaneously administered dose measured 8 h or 24 h after the application. Linear extrapolation suggests that barrier starts to adapt itself to penetrant transport at approximately  $0.75 \text{ mg cm}^{-2}$ .

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**CLAIMS**

1. A method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:
- 10 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
  - 15 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
  - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the
  - 20 diameter of homo-aggregates containing merely the less soluble substance,
  - and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating
  - 25 concentration in the unsolubilised droplet, whichever is higher;
  - and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or
  - 30 of phospholipid bilayers with fluid aliphatic chains,

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- 5     – said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants
- 10    across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.
- 15       2.     The method according to claim 1,  
**characterised in that** the flux across said barrier is increased by enlarging the applied dose per area of said penetrants.
3.     The method according to claims 1 or 2,
- 20    **characterised in that** the pH of the formulation is between 3 and 10, more preferably between 4 and 9, and most preferably between 5 and 8.
4.     The method according to any one of the preceding claims,  
**characterised in that** the formulation comprises:
- 25    – at least one thickening agent in an amount that increases the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of
- 30    oxidation index to less than 100 % per 6 months, more preferably to less

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- 5        than 100 % per 12 months and most preferably to less than 50 % per  
12 months
- and / or at least one microbicide in an amount that reduces the bacterial  
count of 1 million germs added per g of total mass of the formulation to  
less than 100 in the case of aerobic bacteria, to less than 10 in the case of  
10        entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*  
or *Staphilococcus aureus*, after a period of 4 days.

5.        The method according to claim 4,  
**characterised in that** said at least one microbicide is added in an amount  
15        that reduces the bacterial count of 1 million germs added per g of total mass  
of the formulation to less than 100 in the case of aerobic bacteria, to less  
than 10 in the case of entero-bacteria, and to less than 1 in the case of  
*Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 3 days,  
and more preferably after a period of 1 day.

20        6.        The method according to claim 4,  
**characterised in that** said thickening agent is selected from the class of  
pharmaceutically acceptable hydrophilic polymers, such as partially  
etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-,  
25        hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely  
synthetic hydrophilic polymers such as polyacrylates, polymethacrylates,  
poly(hydroxyethyl)-, poly(hydroxypropyl)-,  
poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-  
sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,  
30        polyethylene glycol-lactides, polyethylene glycol-diacrylates,  
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),

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5 poly(propylene fumarate-co-ethylene glycols), poloxamers,  
polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;  
natural gums comprising alginates, carrageenans, guar-gums, gelatines,  
tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;  
mixtures and further derivatives or co-polymers thereof and / or other  
10 pharmaceutically, or at least biologically, acceptable polymers.

7. The method according to claim 6,  
**characterised in that** the concentration of said polymer is in the range  
between 0.01 w-% and 10 w-%, more preferably in the range between 0.1  
15 w-% and 5 w-%, even more preferably in the range between 0.25 w-% and  
3.5 w-% and most preferably in the range between 0.5 w-% and 2 w-%.

8. The method according to claim 4,  
**characterised in that** said anti-oxidant is selected from synthetic phenolic  
20 antioxidants, such as butylated hydroxyanisol (BHA), butylated  
hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548,  
HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.),  
tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-  
trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine,  
25 p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol,  
tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol,  
hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid,  
quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic  
acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta,  
30 gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.  
-acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other

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- 5 suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin,
- 10 diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate,
- 15 probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral;
- 20 endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids
- 25 (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters; caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon,
- 30 sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsoic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid;

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5 oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

10

9. The method according to claim 8, characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 15 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably 20 is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 25 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cysteine is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 30 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between



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5 0.001–0.2 w-%, and most preferably is between 0.005–0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most  
10 preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01–0.2, most typically between 0.3 and 2 w-%.

15 10. The method according claim 4,  
**characterised in that** said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xyleneol,  
20 dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts,  
25 e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

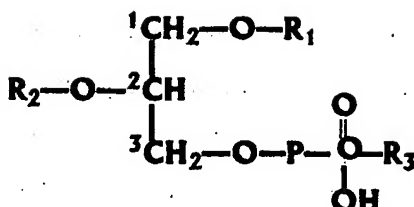
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5           11.    The method according to claim 10,  
          **characterised in that** the bulk concentration of short chain alcohols in the  
          case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more  
          preferably is up to 5 w-%, and most preferably is in the range between  
          0.5-3 w-%, and in the case of chlorobutanol is in the range between  
10   0.3-0.6 w-%; bulk concentration of parabens, especially in the case of  
          methyl paraben is in the range between 0.05-0.2 w-%, and in the case of  
          propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration  
          of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of  
          benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of  
15   phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk  
          concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

          12.    The method according to any one of the preceding claims,  
          **characterised in that** the less soluble amongst the aggregating substances is  
20   a lipid or lipid-like material, especially a polar lipid, whereas the substance  
          which is more soluble in the suspending liquid and which lowers the  
          average elastic energy of the droplet is a surfactant or else has surfactant-  
          like properties and / or is a form of said lipid or lipid-like material which is  
          comparably soluble as said surfactant or the surfactant-like material.

25           13.    Formulation according to claim 12,  
          **characterised in that** the lipid or lipid-like material is a lipid or a lipid  
          from a biological source or a corresponding synthetic lipid or any of its  
          modifications, said lipid preferably belonging to the class of pure  
30   phospholipids corresponding to the general formula



5

where R<sub>1</sub> and R<sub>2</sub> is an aliphatic chain, typically a C<sub>10-20</sub>-acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain; and where R<sub>3</sub> is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C<sub>1-4</sub>-alkyl, C<sub>1-5</sub>-alkyl substituted with carboxy, C<sub>2-5</sub>-alkyl substituted with hydroxy, C<sub>2-5</sub>-alkyl substituted with carboxy and hydroxy, or C<sub>2-5</sub>-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

- 5           14. Formulation according to claim 12,  
characterised in that the surfactant or surfactant-like material is a nonionic,  
a zwitterionic, an anionic or a cationic surfactant; especially a fatty-acid or -  
alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a  
monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate,  
10 taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-  
aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-  
methylglucamide, N- alkyl-N,N- dimethylglycine, 3-  
(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a  
polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-  
15 octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl  
ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-  
isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether,  
polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-  
monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate  
20 (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-  
lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-  
4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the  
corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -  
laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-  
25 monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-  
or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-  
methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate,  
sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium  
taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium  
30 laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-  
glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-

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5 acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,  
or -phosphorylserine, n-tetradecyl- glycero-phosphatidic acid, -  
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,  
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain  
phospholipid, or else a surface-active polypeptide.

10

15. The method according to any of the preceding claims,  
**characterised in that** the average diameter of the penetrant is between  
30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more  
preferably between 50 nm and 200 nm and particularly preferably between  
15 60 nm and 150 nm.

16. The method according to any one of the preceding claims,  
**characterised in that** the total dry weight of droplets in a formulation is  
0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably  
20 between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and  
20 w-%.

17. The method according to any one of the preceding claims,  
**characterised in that** the total dry weight of droplets in a formulation is  
25 selected to increase the formulation viscosity to maximally 5 Nm/s, more  
preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that  
formulation spreading-over and drug retention at the application area is  
enabled.

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5           18. The method according to any one of the preceding claims,  
characterised in that at least one edge-active substance or surfactant and/or,  
at least one amphiphilic substance; and / or at least one hydrophilic fluid and  
the agent are mixed, if required separately, to form a solution, the resulting  
(partial) mixtures or solutions are then combined subsequently to induce,  
10 preferably by action of mechanical energy such as shaking, stirring,  
vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,  
or filtration using convenient driving pressure, the formation of penetrants  
that associate with and / or incorporate the agent

15           19. The method of claim 18,  
characterised in that said amphiphilic substances are dissolved in volatile  
solvents, such as alcohols, especially ethanol, or in other pharmaceutically  
acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl  
20 alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400  
D) or glycerol, other pharmaceutically acceptable organic solvents, such as  
undercooled gas, especially supercritical CO<sub>2</sub>, which are then removed,  
especially by evaporation or dilution, prior to making the final preparation.

25           20. The method according to any one of claims 18 or 19,  
characterised in that the formation of said penetrants is induced by the  
addition of required substances into a fluid phase, evaporation from a  
reverse phase, by injection or dialysis, if necessary under the influence of  
30 mechanical stress, such as shaking, stirring, in especially high velocity  
stirring, vibrating, homogenising, ultrasonication, shearing, freezing and

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5 thawing, or filtration using convenient, in especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

21. The method of claim 20,  
characterised in that the formation of said penetrants is induced by  
10 filtration, the filtering material having pores sizes between 0.01  $\mu\text{m}$  and 0.8  $\mu\text{m}$ , more preferably between 0.02  $\mu\text{m}$  and 0.3  $\mu\text{m}$ , and most preferably between 0.05  $\mu\text{m}$  and 0.15  $\mu\text{m}$ , whereby several filters may be used sequentially or in parallel.

15 22. The method according to any one of claims 18 to 21, characterised in that said agents and penetrants are made to associate, at least partly,  
– after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and  
20 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol into the suspending medium,  
– simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

25 23. The method according to any one of the claims 18 to 22, characterised in that said penetrants, with which the agent is associated, are prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

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5           24.    The method according to any one of the preceding claims,  
**characterised in that** the formulation is applied by spraying, smearing,  
rolling or sponging on the application area, in particular by using a metered  
sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

10           25.    The method according to any one of the preceding claims,  
**characterised in that** the barrier is a part of a mammalian body and / or a  
plant and preferably is skin and / or at least partly keratinised endothelium  
and / or nasal or any other mucosa.

15           26.    The method according to claim 25,  
**characterised in that**, the area dose of said penetrant is between 0.1 mg per  
square centimetre ( $\text{mg cm}^{-2}$ ) and 40  $\text{mg cm}^{-2}$ , more preferably is between  
0.25  $\text{mg cm}^{-2}$  and 30  $\text{mg cm}^{-2}$  and even more preferably is between  
20 0.5  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$ , in case the penetrant is applied on said skin  
and / or said at least partly keratinised endothelium.

            27.    The method according to claim 25,  
**characterised in that** the area dose of said penetrant is between 0.05 mg per  
25 square centimetre ( $\text{mg cm}^{-2}$ ) and 20  $\text{mg cm}^{-2}$ , more preferably is between  
0.1  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$ ) and even more preferably is between  
0.5  $\text{mg cm}^{-2}$  and 10  $\text{mg cm}^{-2}$ , in the case the penetrant is applied on said  
nasal or other mucosa.

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5           28.    The method according to claim 25,  
              **characterised in that** the area dose of said penetrant is between 0.0001 mg  
              per square centimetre ( $\text{mg cm}^{-2}$ ) and  $0.1 \text{ mg cm}^{-2}$ , more preferably is  
              between  $0.0005 \text{ mg cm}^{-2}$  and  $0.05 \text{ mg cm}^{-2}$  and even more preferably is  
              between  $0.001 \text{ mg cm}^{-2}$  and  $0.01 \text{ mg cm}^{-2}$ , in the case that the penetrant is  
10           applied on plant body, plant leaves or plant needles.

              29.    A kit containing said formulation in an amount which enables  
              the formulation to be applied at the selected dose per area, according to any  
              one of the preceding claims.

15           30.    The kit according to claim 29,  
              **characterised in that** the formulation is contained in a bottle or any other  
              packaging vessel.

20           31.    The kit according to claims 29 or 30,  
              **characterised in that** it contains a device for administering the formulation.

              32.    The kit according to claim 31,  
              **characterised in that** the device is a non-occlusive patch.

25           33.    A device comprising a non-occlusive patch, containing the  
              formulation as in any one of claims 1 to 27 in an amount that yields the dose  
              per area according to any one of the preceding claims.

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- 5           34.    A non-occlusive patch according to claim 33,  
          comprising a laminated composite of:
- a backing layer;
  - an active agent-permeable membrane, the backing layer and membrane  
      defining
  - 10   – a reservoir therebetween that contains the formulation of the active agent,  
      said reservoir having a smaller periphery than the backing layer and  
      membrane such that a portion of the backing layer and membrane extends  
      outwardly of the periphery of the reservoir;
  - a pressure sensitive adhesive layer that underlies and covers the active
  - 15   agent-permeable membrane and said outwardly extending portion of the  
      backing layer and membrane.

35.    A non-occlusive patch according to claim 33,  
          comprising a laminated composite of:
- 20   – a backing layer;
  - a matrix layer that contains the formulation of the active agent; and
  - a pressure sensitive adhesive layer.

36.    The device according to claim 33,
- 25   **characterised in that** the formulation and / or agent and / or  
      suspension / dispersion of penetrants without the agent are kept during the  
      storage in several, more preferably less than 5, even more preferably in 3,  
      and most preferred in less than 3 separate inner compartments of the device  
      which, in case, are combined prior to or during the application of the
- 30   formulation.

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5           37.    The device according to claim 36,  
          **characterised in that** said compartment(s) filled with the formulation  
          and / or agent and / or suspension of penetrants without the agent, is (are)  
          covered, on one or both sides, with a non-occlusive, semi-permeable  
          membrane that lets small molecules, such as water, but only few or not the  
10   penetrants pass.

          38.    The device according to claim 37,  
          **characterised in that** said non-occlusive, semi-permeable membrane is the  
15   same or different, if it is used on both sides of said device.

          39.    The device according to claims 37 or 38,  
          **characterised in that** the water permeability of said semi-permeable but  
          non-occlusive membrane is at least  $10 \text{ mg cm}^{-2} \text{ h}^{-1}$ , more preferably exceeds  
20    $50 \text{ mg cm}^{-2} \text{ h}^{-1}$  and most preferably is greater than  $100 \text{ mg cm}^{-2} \text{ h}^{-1}$ .

          40.    The device according to claims 37 to 39,  
          **characterised in that** the area of said semi-permeable membrane is between  
           $0.5 \text{ cm}^2$  and  $250 \text{ cm}^2$ , more preferably is between  $1 \text{ cm}^2$  and  $100 \text{ cm}^2$ , even  
25   more preferably is between  $2 \text{ cm}^2$  and  $50 \text{ cm}^2$  and most preferred is between  
           $4 \text{ cm}^2$  and  $25 \text{ cm}^2$ .

          41.    The device according to claims 37 to 40,  
          **characterised in that** the area of said semi-permeable membrane is the area  
30   substantially covered by the formulation filled part of the device.

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- 5           42.    The device according to claims 37 to 41,  
characterised in that the penetrant flux across the barrier is controlled by  
the permeability of, or the suspension-medium evaporation across, the  
semi-permeable, non-occlusive membrane
- 10           43.    The device according to claims 37 to 42,  
characterised in that the device is filled with the formulation and / or agent  
molecules and / or suspension of penetrants without agent, either separately  
or together, prior to the administration of said patch, preferably 360 min,  
more preferably 60 min, even more preferably 30 min and most  
15    preferably within few minutes before placing the device on the barrier..
44.    A method of administering an agent onto a mammalian body  
or a plant, for transporting said agent through a barrier, such as the intact  
20    skin/mucosa or cuticle, respectively, when the agent is associated with the  
penetrant which is capable of transporting said agent through the skin pores  
or through the passages in mucosa or cuticle, or else is capable of enabling  
agent permeation through skin pores after said penetrant has opened and/or  
entered said pores, comprising the steps of:
- 25    — preparing a formulation by suspending or dispersing said penetrants in a  
polar liquid in the form of fluid droplets surrounded by a membrane-like  
coating of one or several layers, said coating comprising at least two  
kinds or forms of amphiphilic substances with a tendency to aggregate,  
provided that
- 30    — said at least two substances differ by at least a factor of 10 in solubility in  
said polar liquid,

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- 5    – and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
- 10   – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
- and / or the presence of the more soluble substance lowers the average  
15   elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said  
20   barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- 25   – applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

45:   The method according to claim 44,  
**characterised in that** the flux of penetrants across said barrier is increased  
30   by enlarging the applied dose per area of said penetrants.

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5           46.    The method according to claims 44 or 45,  
              **characterised in that** the pH of the formulation is between 3 and 10, more  
              preferably between 4 and 9, and most preferably between 5 and 8.

              47.    The method according to claims 44 to 46,  
10   **characterised in that** the formulation comprises:  
              – at least one thickening agent in an amount that increases the formulation  
              viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most  
              preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug  
              retention at the application area is enabled,  
15   – and / or at least one antioxidant in an amount that reduces the increase of  
              oxidation index to less than 100 % per 6 months, more preferably to less  
              than 100 % per 12 months and most preferably to less than 50 % per  
              12 months  
              – and / or at least one microbicide in an amount that reduces the bacterial  
20   count of 1 million germs added per g of total mass of the formulation to  
              less than 100 in the case of aerobic bacteria, to less than 10 in the case of  
              entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*  
              or *Staphylococcus aureus*, after a period of 4 days.

25           48.    Formulation according to claim 47,  
              **characterised in that** said at least one microbicide is added in an amount  
              that reduces the bacterial count of 1 million germs added per g of total mass  
              of the formulation to less than 100 in the case of aerobic bacteria, to less  
30   than 10 in the case of entero-bacteria, and to less than 1 in the case of

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- 5    *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days,  
and more preferably after a period of 1 day.

49.    The method according to claim 47,  
**characterised in that** said thickening agent is selected from the class of  
10    pharmaceutically acceptable hydrophilic polymers, such as partially  
etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-,  
hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely  
synthetic hydrophilic polymers such as polyacrylates, polymethacrylates,  
poly(hydroxyethyl)-, poly(hydroxypropyl)-,  
15    poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-  
sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,  
polyethylene glycol-lactides, polyethylene glycol-diacrylates,  
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),  
poly(propylene fumarate-co-ethylene glycols), poloxamers,  
20    polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;  
natural gums comprising alginates, carrageenans, guar-gums, gelatines,  
tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;  
mixtures and further derivatives or co-polymers thereof and / or other  
pharmaceutically, or at least biologically, acceptable polymers.

25

50.    The method according to claim 49,  
**characterised in that** the concentration of said polymer is in the range  
between 0.01 w- % and 10 w- %, more preferably in the range between 0.1  
w- % and 5 w- %, even more preferably in the range between 0.25 w- % and  
30    3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

- 5           51. The method according to claim 47,  
characterised in that said anti-oxidant is selected from synthetic phenolic  
antioxidants, such as butylated hydroxyanisol (BHA), butylated  
hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548,  
HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.),  
10   tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-  
trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine,  
p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol,  
tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol,  
hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid,  
15   quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic  
acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta,  
gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.  
-acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other  
suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and  
20   corresponding amide- and thiocarboxamide analogues; ascorbic acid and its  
salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g.  
6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.);  
non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin,  
diclofenac, mefenamic acid, flufenamic acid, phenylbutazone,  
25   oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen,  
ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine,  
quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital,  
acetaminophen); aminosalicylic acids and derivatives; methotrexate,  
probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol),  
30   ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as  
nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor



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5 blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes  
 10 with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g.  
 15 coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsoic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers,  
 20 sulphoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides; or else is an oxidation suppressing enzyme.

25 52. The method according to claim 51, characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most  
 30 preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most

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5 preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is  
10 between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most  
15 preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most  
20 typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and  
25 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

53. The method according claim 47,  
30 **characterised in that** said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol,

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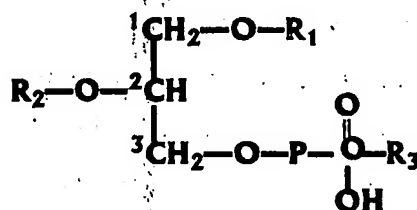
- 5 chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts;
- 10 quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its
- 15 gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

54. The method according claim 53,
- characterised in that the bulk concentration of short chain alcohols in the**
- 20 **case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of**
- 25 **propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.**

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5           55.    The method according to claims 44 to 54,  
               **characterised in that** the less soluble amongst the aggregating substances is  
               a lipid or lipid-like material, especially a polar lipid, whereas the substance  
               which is more soluble in the suspending liquid and which lowers the  
               average elastic energy of the droplet is a surfactant or else has surfactant-  
 10       like properties and / or is a form of said lipid or lipid-like material which is  
               comparably soluble as said surfactant or the surfactant-like material.

              56.    Formulation according to claim 55,  
               **characterised in that** the lipid or lipid-like material is a lipid or a lipid  
 15       from a biological source or a corresponding synthetic lipid or any of its  
               modifications, said lipid preferably belonging to the class of pure  
               phospholipids corresponding to the general formula



20       where R<sub>1</sub> and R<sub>2</sub> is an aliphatic chain, typically a C<sub>10-20</sub>-acyl, or -alkyl or  
       partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-,  
       elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-,  
       myristoyl-, palmitoyl-, or stearoyl chain; and where R<sub>3</sub> is hydrogen,  
 25       2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C<sub>1,4</sub>-alkyl, C<sub>1,5</sub>-alkyl substituted  
       with carboxy, C<sub>2,5</sub>-alkyl substituted with hydroxy, C<sub>2,5</sub>-alkyl substituted with

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5 carboxy and hydroxy, or C<sub>2-5</sub>-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the  
10 group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or  
15 synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

20 57. Formulation according to claim 55,  
**characterised in that** the surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate,  
25 glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-  
30 octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-

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5 isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethyleneglykol-20-monolaurate (Tween 20) or polyethyleneglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-  
 10 4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-  
 15 methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-  
 20 acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl- glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

25

58. The method according to claims 44 to 57,  
 characterised in that the average diameter of the penetrant is between  
 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more  
 preferably between 50 nm and 200 nm and particularly preferably between  
 30 60 nm and 150 nm.

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5           59.    The method according to claims 44 to 58,  
characterised in that the total dry weight of droplets in a formulation is  
0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably  
between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and  
20 w-%.

10           60.    The method according to claims 44 to 59,  
characterised in that the total dry weight of droplets in a formulation is  
selected to increase the formulation viscosity to maximally 5 Nm/s, more  
preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that  
15   formulation spreading-over and drug retention at the application area is  
enabled.

          61.    The method according to claims 44 to 60,  
characterised in that at least one edge-active substance or surfactant and/or  
20   at least one amphiphilic substance, and / or at least one hydrophilic fluid and  
the agent are mixed, if required separately, to form a solution, the resulting  
(partial) mixtures or solutions are then combined subsequently to induce,  
preferably by action of mechanical energy such as shaking, stirring,  
vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,  
25   or filtration using convenient driving pressure, the formation of penetrants  
that associate with and / or incorporate the agent

          62.    The method according to claim 61,  
characterised in that said amphiphilic substances are dissolved in volatile  
30   solvents, such as alcohols, especially ethanol, or in other pharmaceutically  
acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl

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5 alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO<sub>2</sub>, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

10 63. The method according to any one of claims 61 or 62, **characterised in that** the formation of said penetrants is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring,  
15 vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

20 64. The method according to claim 63, **characterised in that** the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used  
25 sequentially or in parallel.

65. The method according to any one of claims 45 to 64, **characterised in that** said agents and penetrants are made to associate, at least partly,  
30 – after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and



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- 5        2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol  
         (molecular weight: 200-400 D) or glycerol into the suspending medium,  
– simultaneously with penetrant formation, if required using the drug  
         co-solution and, at least some, penetrant ingredients.

10            66.    The method according to any one of the claims 45 to 65,  
         **characterised in that** said penetrants, with which the agent is associated,  
         are prepared immediately before the application of the formulation, if  
         convenient, from a suitable concentrate or a lyophilisate.

15            67.    The method according to any one of the claims 45 to 66,  
         **characterised in that** the formulation is applied by spraying, smearing,  
         rolling or sponging on the application area, in particular by using a metered  
         sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

20            68.    The method according to any one of the claims 45 to 67,  
         **characterised in that** the barrier is skin or at least partly keratinised  
         endothelium and / or nasal or any other mucosa.

                 69.    The method according to claim 68,  
25   **characterised in that**, the area dose of said penetrant is between 0.1 mg per  
         square centimetre ( $\text{mg cm}^{-2}$ ) and 40  $\text{mg cm}^{-2}$ , more preferably is between  
         0.25  $\text{mg cm}^{-2}$  and 30  $\text{mg cm}^{-2}$  and even more preferably is between  
         0.5  $\text{mg cm}^{-2}$  and 15  $\text{mg cm}^{-2}$ , in the case that the penetrant is applied on  
         said skin and / or said at least partly keratinised endothelium.

30

5           70.    The method according to claim 68,  
              **characterised in that** the area dose of said penetrant is between 0.05 mg per  
              square centimetre ( $\text{mg cm}^{-2}$ ) and  $20 \text{ mg cm}^{-2}$ , more preferably is between  
               $0.1 \text{ mg cm}^{-2}$  and  $15 \text{ mg cm}^{-2}$  and even more preferably is between  
               $0.5 \text{ mg cm}^{-2}$  and  $10 \text{ mg cm}^{-2}$ , in the case that the penetrant is applied on  
10       said nasal or other mucosa.

              71.    The method according to claim 68,  
              **characterised in that** the area dose of said penetrant is between  $0.0001 \text{ mg}$   
              per square centimetre ( $\text{mg cm}^{-2}$ ) and  $0.1 \text{ mg cm}^{-2}$ , more preferably is  
15       between  $0.0005 \text{ mg cm}^{-2}$  and  $0.05 \text{ mg cm}^{-2}$  and even more preferably is  
              between  $0.001 \text{ mg cm}^{-2}$  and  $0.01 \text{ mg cm}^{-2}$ , in the case that the penetrant is  
              applied on plant body, plant leaves or plant needles.

              72.    The method of claim 44, used for generating an immune  
20       response on a human or other mammal by vaccinating said mammal.

              73.    The method of claim 44, used for generating a therapeutic  
              effect in a human or other mammal.

25           74.    The method of claim 44 for the treatment of inflammatory  
              disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration  
              syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-  
              haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic  
              anemia, macroglobulinaemia, thrombocytopenic purpura, furthermore, for the  
30       management of bone disorders, cerebral oedema, Cogan's syndrome,  
              congenital adrenal hyperplasia, connective tissue disorders, such as lichen,

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- 5 lupus erythematosus, polymyalgia rheumatica, polymyositis and  
dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves'  
ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal  
vasculitis, scleritis, for some gastro-intestinal disorders, such as  
inflammatory bowel disease, nausea and oesophageal damage, for  
10 hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis),  
for Kawasaki disease, myasthenia gravis, various pain syndromes, such as  
postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory  
disorders, such as asthma, for the management of rheumatoid disease and  
osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema,  
15 erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis,  
pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

20

25

30

Figure 1

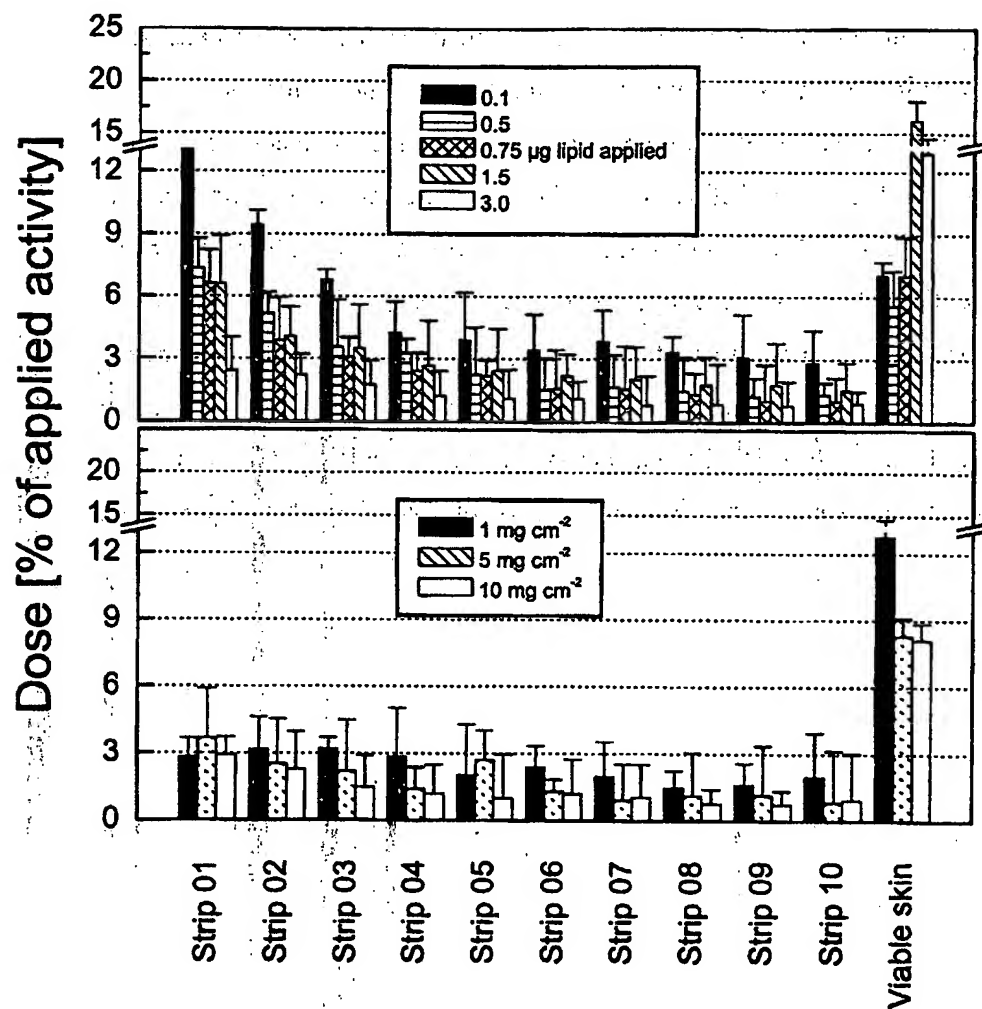


Figure 2

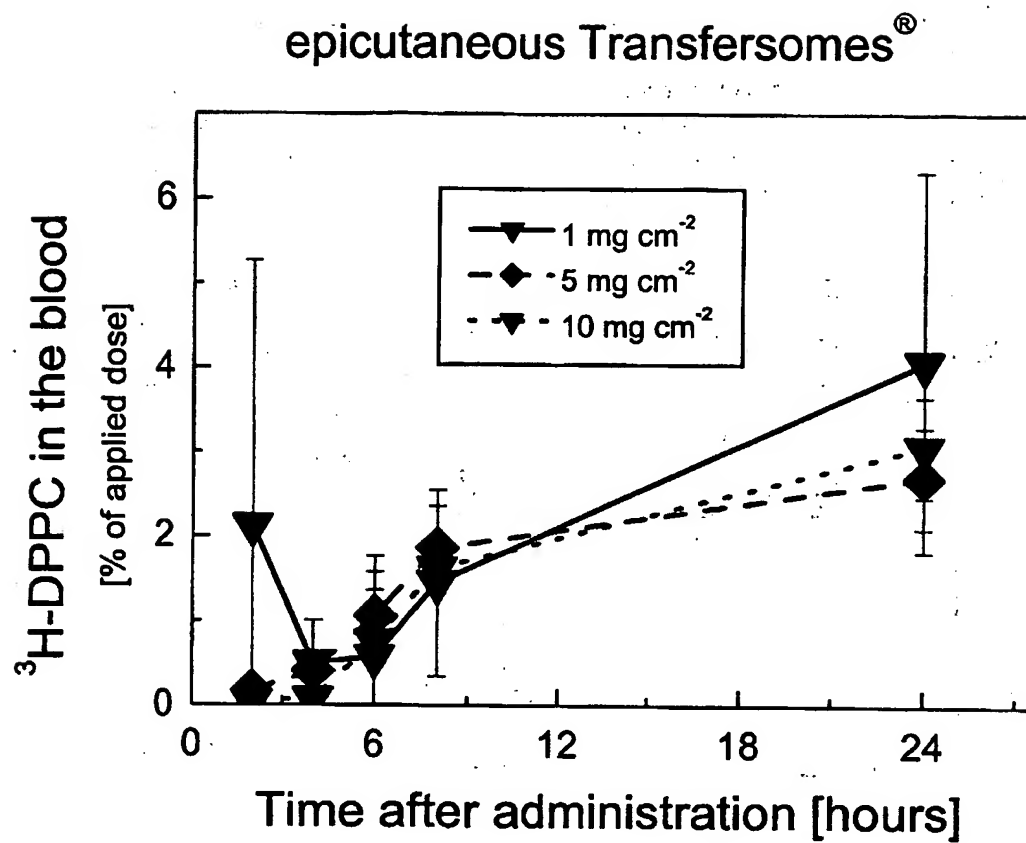


Figure 3

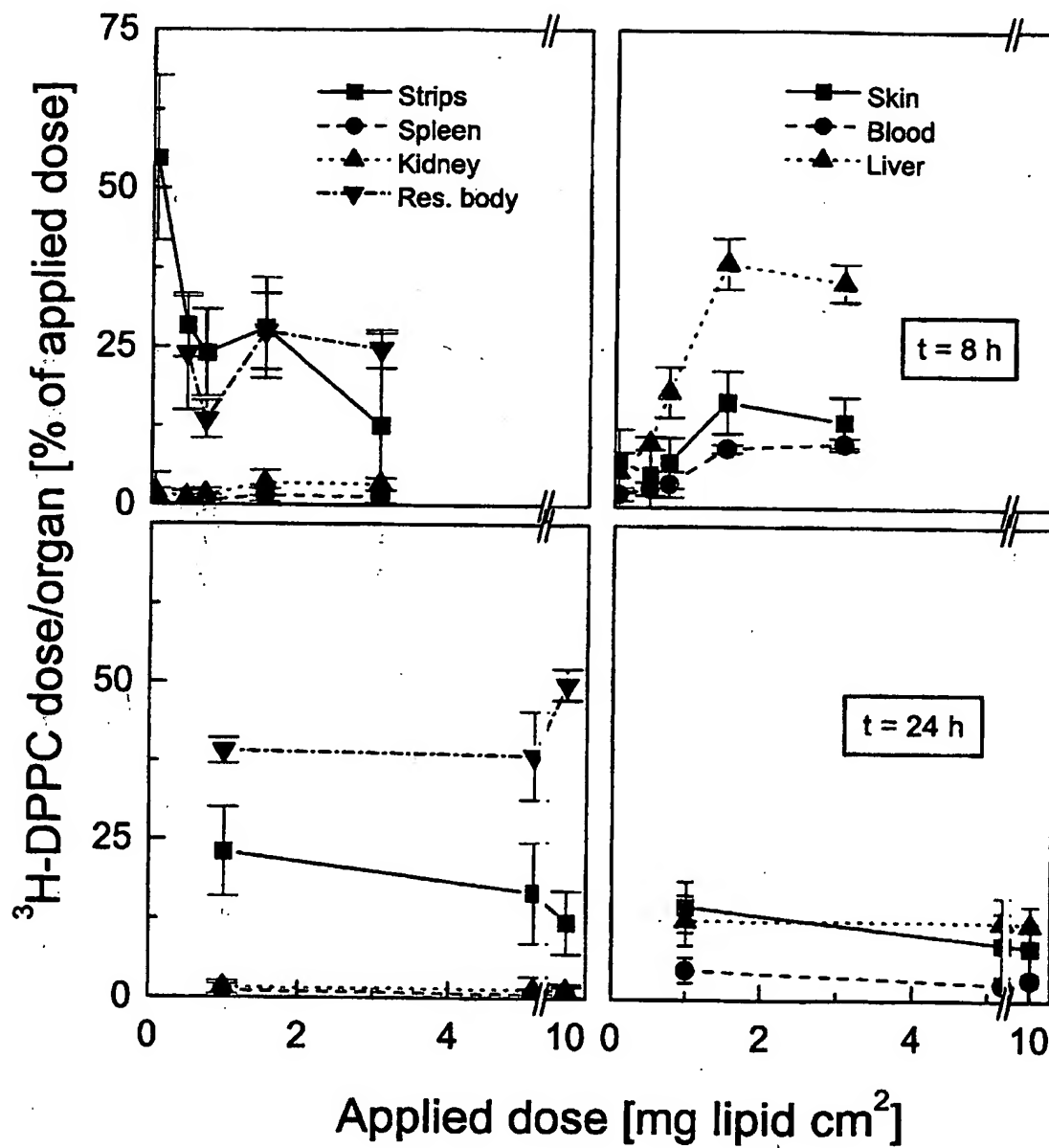


Figure 4

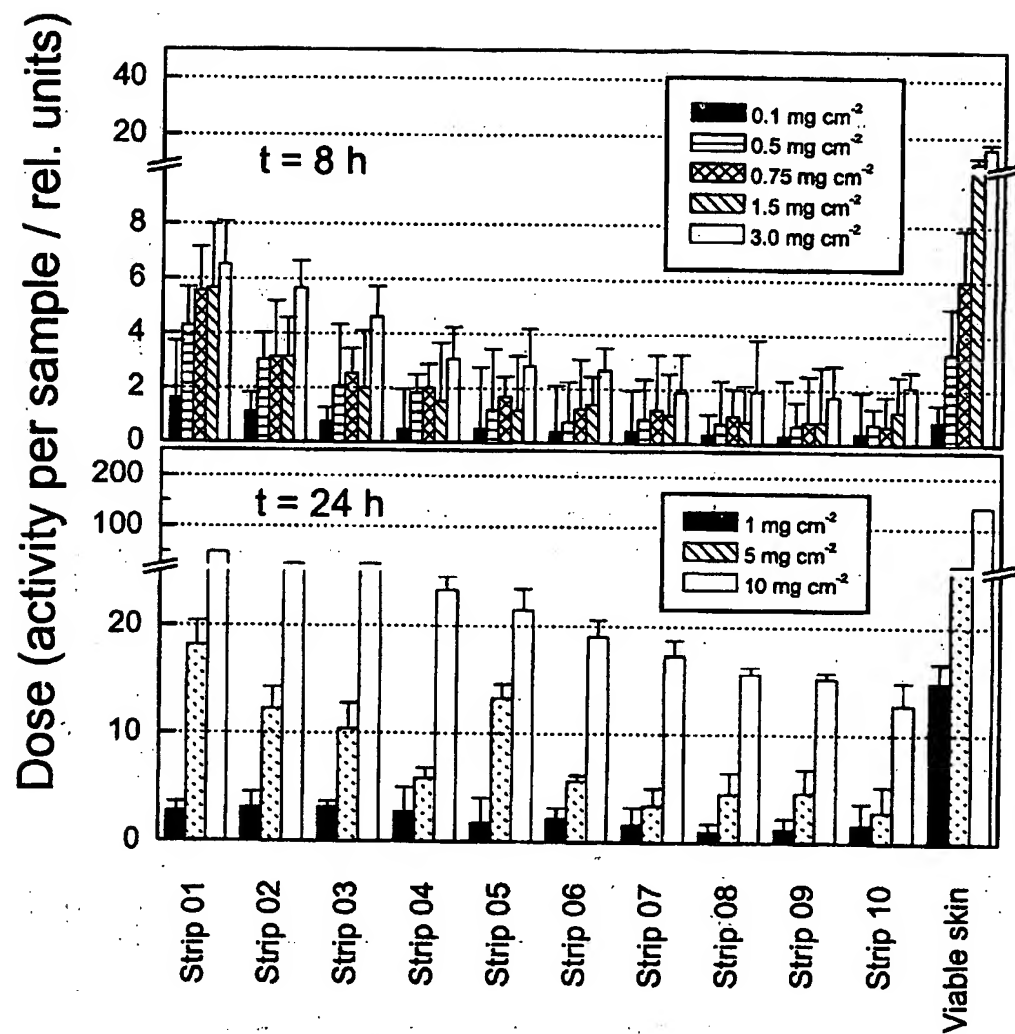


Figure 5

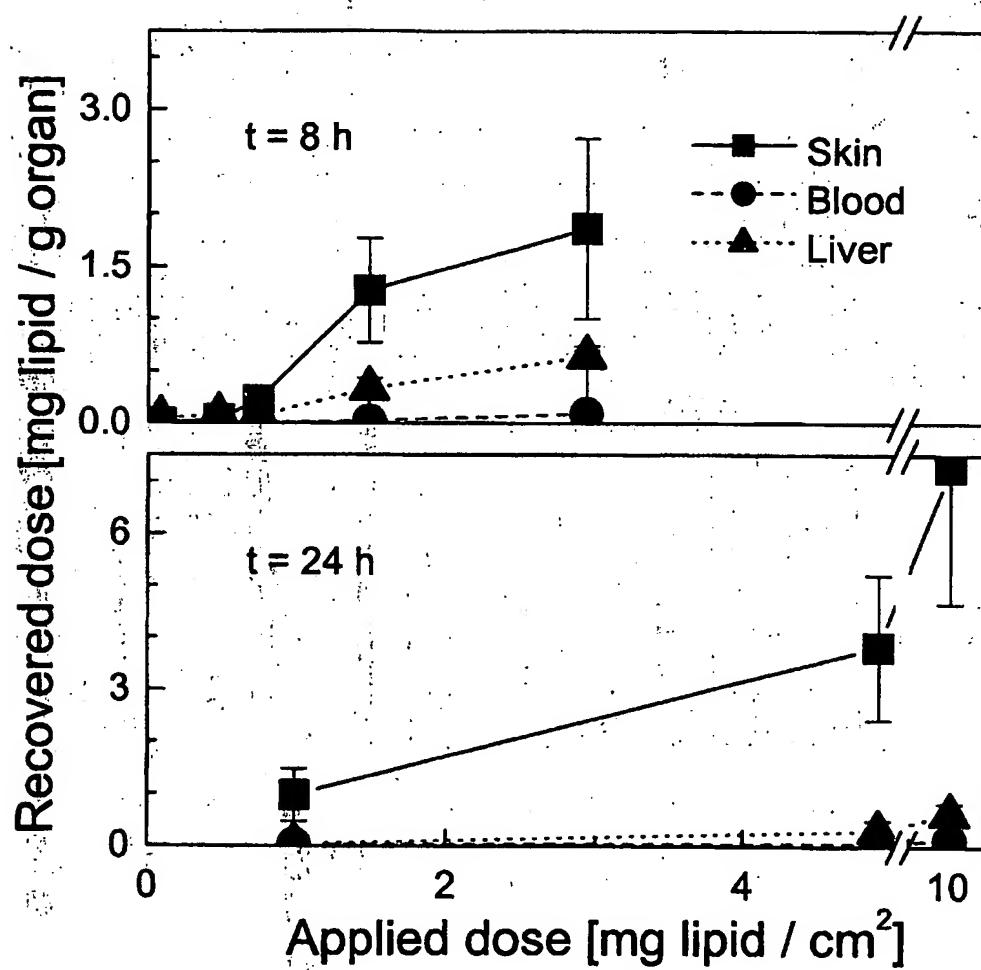




Figure 6

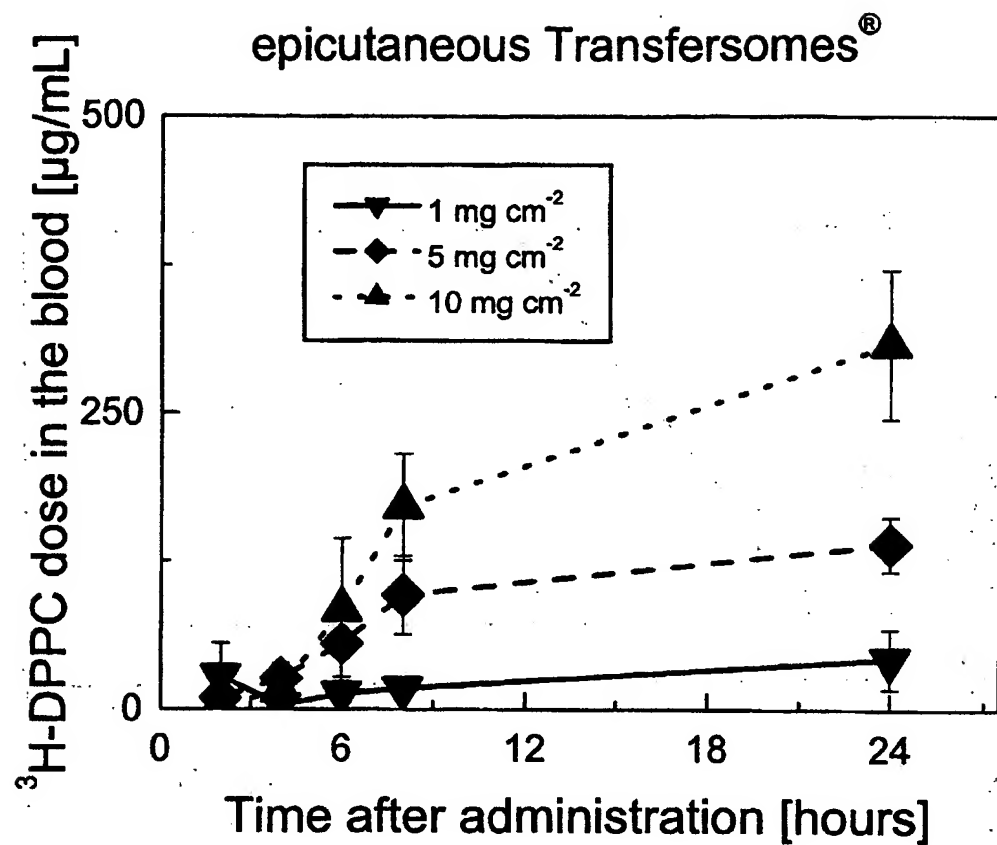
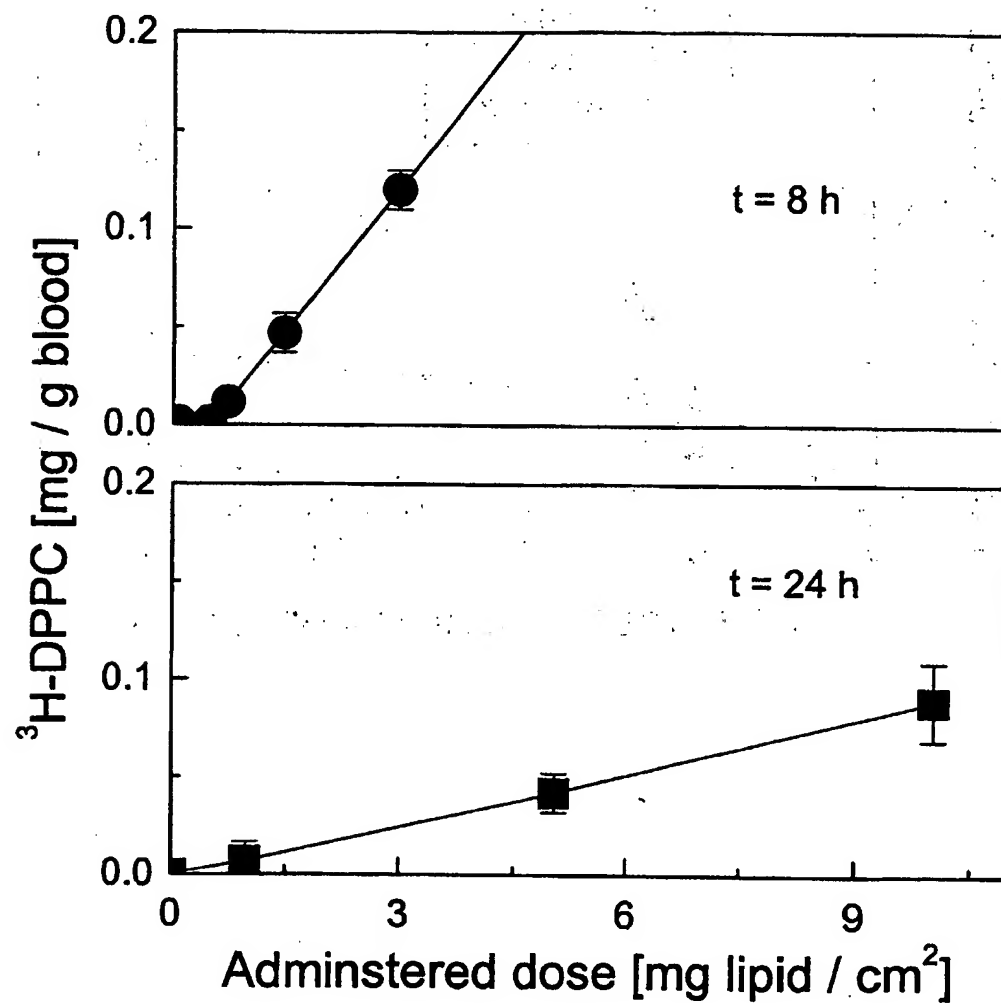


Figure 7



## INTERNATIONAL SEARCH REPORT

Inter. nat. Application No.

PCT/EP 99/04659

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K9/127 A61K9/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. CEVC ET AL.: "transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo" JOURNAL OF CONTROLLED RELEASE, vol. 45, no. 3, 7 April 1997 (1997-04-07), pages 211-226, XP000640528 Amsterdam (nL) page 211, abstract page 225, conclusions page 213, paragraph 2.1.  -/-	1,2, 12-15, 18-29, 44,45, 55-59, 61-65, 68-71, 73,74

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means  
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Date of the actual completion of the international search

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Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

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PCT/EP 99/04659

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17255 A (CEVC) 30 April 1998 (1998-04-30)  the whole document page 19, line 21 - line 25 page 21, line 16 - page 23, line 17 page 24, line 9 - line 25 page 27, line 4 - page 28, line 4 claim 18	1-28, 44-47, 51, 53, 55-59, 61-66, 68-74
A	V.M. KNEPP ET AL.: "controlled drug release from a novel liposomal delivery system. II. transdermal delivery characteristics" JOURNAL OF CONTROLLED RELEASE, vol. 12, no. 1, March 1990 (1990-03), pages 25-30, XP000113393 Amsterdam (NL) page 26, column 1, paragraph 6. page 26, column 2, paragraph 2	29, 31
A	EP 0 674 913 A (LECTEC CORPORATION) 4 October 1995 (1995-10-04) the abstract	1, 29, 32
A	WO 98 30215 A (CILAG) 16 July 1998 (1998-07-16) claims 1-9	4-11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 99/04659

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 1-12, 15-28, 44-55, 58-71 (all partially) and 72-74 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/04659

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9817255 A	30-04-1998	AU 4510897 A	15-05-1998
		EP 0935457 A	18-08-1999
EP 674913 A	04-10-1995	US 5536263 A	16-07-1996
		AU 676623 B	13-03-1997
		AU 1002495 A	12-10-1995
		CA 2133598 A	01-10-1995
		FI 950465 A	01-10-1995
		JP 7265353 A	17-10-1995
		NO 951217 A	02-10-1995
		US 5741510 A	21-04-1998
WO 9830215 A	16-07-1998	AU 5777498 A	03-08-1998